

Amendments to the Claims

1-19 (Canceled)

20. (Currently amended) A ~~plasmid having a nucleic acid molecule~~ sequence region comprising an open reading frame encoding a cleavable single-chain polypeptide, said open reading frame comprising:

- a) a first nucleotide sequence ~~region comprising~~ encoding at least a portion of a clostridial neurotoxin heavy chain binding element able to preferentially interact with a target cell surface marker under physiological conditions;
 - i) ~~a first portion encoding a first amino acid sequence region comprising a binding element able to specifically bind a target cell surface marker under physiological conditions; and~~
- b) a second nucleotide sequence encoding at least a portion of a clostridial neurotoxin heavy chain translocation element able to facilitate the transfer of said single-chain polypeptide across a vesicular membrane;
 - ii) ~~a second portion encoding a second amino acid sequence region comprising a translocation element able to facilitate the transfer of a polypeptide across a vesicular membrane;~~
- [[b]]c) a ~~second~~third nucleotide sequence ~~region~~ encoding a third amino acid sequence region comprising at least a portion of a therapeutic element peptide having biological activity when released into the cytoplasm of the target cell, and
- d) a fourth nucleotide sequence encoding a peptide comprising a non-native Clostridial neurotoxin protease cleavage site;

wherein said fourth nucleotide sequence intervenes between said second sequence and said third nucleotide sequence.

~~wherein said first and second nucleotide sequence regions are separated by a third nucleotide sequence region encoding a fourth amino acid sequence comprising a protease cleavage site which is cleaved when exposed to a protease, provided said third amino acid sequence region is not cleaved by a human protease or a protease normally expressed by a cell expressing said single-chain polypeptide, and wherein said single-chain polypeptide is expressed by said plasmid within a suitable host cell.~~

21. (Currently amended) The ~~plasmidmolecule~~ of claim [[20]]20, wherein said ~~first or second nucleotide sequence region~~open reading frame further comprises a fifth nucleotide sequence encoding ~~encodes~~ an amino acid sequence region ~~a peptide comprising~~ a target-binding portion of a binding tag.

22. (Currently amended) The ~~plasmidmolecule~~ of claim [[21]]21, wherein said binding tag comprises a target-binding portion of a polypeptide selected from the group consisting

~~efcomprises a His₆, a monoclonal antibodiesantibody, a maltose binding protein, a glutathione-S-transferase, a protein A, andor a calmodulin binding protein.~~

23. (Currently amended) The plasmidmolecule of claim [[20]]20, wherein said ~~first nucleotide sequence region encodes at least a portion of~~binding element is a Clostridium botulinum neurotoxin heavy chain a clostridial neurotoxin heavy chain.
24. (Currently amended) The plasmidmolecule of claim [[23]]20, wherein said ~~first nucleotide sequence region encodes at least a portion of~~translocation element is a Clostridium botulinum neurotoxin heavy chain.
25. (Currently amended) The plasmidmolecule of claim [[23]]20, wherein said ~~first nucleotide sequence region encodes at least a portion of~~translocation element is a Clostridium tetani neurotoxin heavy chain.
26. (Currently amended) The plasmidmolecule of either of claim 20 or 2320, wherein said ~~second nucleotide sequence region encodes at least a portion of~~therapeutic element peptide comprises a clostridial neurotoxin light chain.
27. (Currently amended) The plasmidmolecule of claim [[26]]26, wherein said ~~second nucleotide sequence region encodes at least a portion of~~clostridial neurotoxin light chain is a Clostridium botulinum neurotoxin light chain.
28. (Currently amended) The plasmidmolecule of claim [[26]]26, wherein said ~~second nucleotide sequence region encodes at least a portion of~~clostridial neurotoxin light chain is a Clostridium tetani neurotoxin light chain.
- 29-31. (Canceled)
32. (Currently amended) A method of making a cleavable single-chain polypeptide ~~derived from a clostridial neurotoxin comprising:~~
 - a) inserting the plasmid of any one of claims 20-25 or 29-3120-28, 31 or 38 into a suitable host cell,
 - b) growing said host cell in culture, and
 - c) permitting or inducing the host cell to express the single chain polypeptide encoded by said plasmid.
33. (Currently amended) A method of purifying a recombinantcleavable single chain polypeptide ~~derived from a clostridial neurotoxin comprising:~~
 - a) lysing a host cell expressing a single chain polypeptide from the plasmid of either of claim 21 or 22 to produce a cell lysate,
 - b) contacting said cell lysate with a target compound so as to form a specific binding complex capable of being immobilized comprising said binding tag and said target compound, and
 - c.) separating said binding complex from said cell lysate.

34-37. (Canceled)

38. (New) The molecule of claim 20, wherein said binding element is a *Clostridium tetani* neurotoxin heavy chain.
39. (New) The molecule of claim 20, wherein said protease cleavage site comprising SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 22 or SEQ ID NO: 23.
40. (New) A nucleic acid molecule comprising an open reading frame encoding a cleavable single-chain polypeptide, said open reading frame comprising:
 - a) a first nucleotide sequence encoding at least a portion of a binding element peptide able to preferentially interact with a sensory afferent neuron cell surface marker under physiological conditions;
 - b) a second nucleotide sequence encoding at least a portion of a clostridial neurotoxin heavy chain translocation element able to facilitate the transfer of said single-chain polypeptide across a vesicular membrane;
 - c) a third nucleotide sequence encoding at least a portion of a clostridial neurotoxin light chain therapeutic element having biological activity when released into the cytoplasm of said target cell; and
 - d) a fourth nucleotide sequence encoding a peptide comprising a non-native Clostridial neurotoxin protease cleavage site;wherein said fourth nucleotide sequence intervenes between said second sequence and said third nucleotide sequence.
41. (New) The molecule of claim 40, wherein said open reading frame further comprises a fifth nucleotide sequence encoding a peptide comprising a target-binding portion of a binding tag.
42. (New) The molecule of claim 41, wherein said target-binding portion comprises a His₆, a monoclonal antibody, a maltose binding protein, a glutathione-S-transferase, a protein A or a calmodulin binding protein.
43. (New) The molecule of claim 40, wherein said translocation element is a *Clostridium botulinum* neurotoxin heavy chain.
44. (New) The molecule of claim 40, wherein said translocation element is a *Clostridium tetani* neurotoxin heavy chain.
45. (New) The molecule of claim 40, wherein said therapeutic element is a *Clostridium botulinum* neurotoxin light chain.
46. (New) The molecule of claim 40, wherein said therapeutic element is a *Clostridium tetani* neurotoxin light chain.

Serial No.: 09/648,692 Activatable Recombinant Neurotoxin

Filed: August 25, 2000

47. (New) The molecule of claim 40, wherein said protease cleavage site comprising SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 22 or SEQ ID NO: 23.